FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **101195-63**

U.S APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/937126

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE00/00927	**23 March 2000 (23.03.00)**	**26 March 1999 (26.03.99)**

TITLE OF INVENTION
IMMUNOADSORBER FOR USE IN SEPSIS THERAPY

APPLICANT(S) FOR DO/EO/US
Hans-Werner Heinrich, Hans-Jurgen Hahn, Udo Meyer, Peter Kruschke and Heinz-Jurgen WAGNER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

U.S. APPLICATION NO (IF KNOWN, SEE 3	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/937126	PCT/DE00/00927	101195-63

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1000.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	17 - 20 =	0	x $18.00	**$0.00**	
Independent claims	1 - 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$860.00**	
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$430.00**	
SUBTOTAL =				**$430.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$430.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$430.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **14-1263** in the amount of **$430.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **14-1263** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Bruce S. Londa
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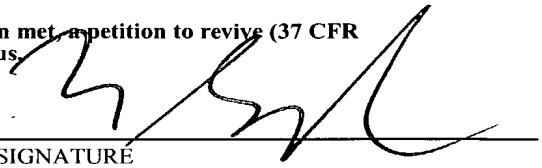

SIGNATURE

Bruce S. Londa
NAME

33,531
REGISTRATION NUMBER

September 20, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty's Docket No.: 101195-63

EXAMINER : TBA

GROUP ART UNIT : TBA

APPLICANT : Hans-Werner Heinrich

APPLN. NUMBER : 09/937,126

FILED : TBA

FOR : Immunoadsorber for Use in Sepsis Therapy

SUBMISSION OF "SEQUENCE LISTING," COMPUTER READABLE COPY, AND/OR AMENDMENT PERTAINING THERETO FOR BIOTECHNOLOGY INVENTION CONTAINING NUCLEOTIDE AND/OR AMINO ACID SEQUENCE

Box PCT
Hon. Assistant Commissioner of Patent
Washington, D.C. 20231

Sir:

1. This is in response to the Office Communication mailed March 5, 2002, a copy of which is enclosed.

IDENTIFICATION OF DECLARANT

2. I, Bruce S. Londa, state the following:

ITEMS BEING SUBMITTED

3. Submitted herewith is/are

a) "Sequence Listing(s)" for the nucleotide and/or amino acid sequence(s) in this application. Each "Sequence Listing" is assigned a separate identifier as required in 37 CFR 1.821(c) and 37 CFR 1.822 and 1.823.

b) An amendment to the description and/or claims, wherein reference is made to the sequence by use of the assigned identifier, as required in 37 CFR 1.821(d).

c) A copy of each "Sequence Listing" submitted for this application in computer readable form, in accordance with the requirements of 37 1.821(e) and 1.824.

d) A statement that the content of each "Sequence Listing" submitted and each computer readable copy are the same, as required in 37 CFR 1.821(g).

e) Because this submission is made in fulfilling the requirement under 37 CFR 1.821(g), a statement that the submission includes no new matter.

STATEMENT THAT "SEQUENCE LISTING"
AND COMPUTER READABLE COPY ARE THE SAME
AND/OR THAT PAPERS SUBMITTED INCLUDES NO NEW MATTER

4. I hereby state that each computer readable form submitted in this application, including those forms requested to be transferred from applicant's other application, is the same as the "Sequence Listing" to which it is indicated to relate. All papers accompanying this submission, or for which a request for transfer from applicant's other application, introduce no new matter.

STATUS

6. Applicant qualifies for small entity.

AMENDMENT

7. In the specification, please add the "Sequence Listing" set forth on the attached sheets.

FEE DEFICIENCY

8. A petition for a one month extension of the term is enclosed. The Commissioner is hereby authorized to charge any additional fees which may be required to make this response timely, or credit any overpayment, to Deposit Account No. 14-1263.

Respectfully Submitted,

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Attorney Docket No. : 101195-63

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	Hans-Werner HEINRICH, Hans-Jürgen HAHN, Udo MEYER, Peter KRUSCHKE and Heinz-Jürgen WAGNER
PCT Application No.:		PCT/DE00/00927
Serial No.	:	To Be Assigned
Filed	:	Herewith
For	:	IMMUNOADSORBER FOR USE IN SEPSIS THERAPY
Art Unit	:	To Be Assigned
Examiner	:	To Be Assigned

September 20, 2001

BOX PCT
Hon. Assistant Commissioner For Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

In advance of prosecution, kindly amend the above-identified application as follows and consider the following remarks:

IN THE CLAIMS

Claim 4 (amended). Immunoadsorber according to Claim 1, wherein further antibodies against sepsis mediators are contained as a function of the state of the dysregulation.

Claim 5 (amended). Immunoadsorber according to Claim 1, wherein these antibodies are aimed against TNF, IL1, IL6, IL8 and/or IL10.

Claim 11 (amended). Immunoadsorber according to Claim 1, wherein the organic or synthetic carrier material comprises membranes of particles of polystyrenes, carbohydrates such as cellulose or agarose derivates, or acrylates.

Claim 14 (amended). Method for the production of immunoadsorbers according to Claim 1, wherein antibodies aimed against C3a and/or C5a and LPS and, if need be, against further sepsis mediators are covalently or adsorptively coupled to carrier materials of organic or synthetic polymers.

Please cancel claims 16 and 17.

REMARKS

This Preliminary Amendment is being filed to place the claims into conventional format, and to eliminate improper multiple dependency.

Favorable action is respectfully solicited.

ADDITIONAL FEE

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

Respectfully submitted,

NORRIS, McLAUGHLIN & MARCUS, P.A.

By _____

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I hereby certify that this paper is being deposited with the United States Postal Service as Express Mail, Label No. EL867734725US to BOX PCT, The Hon. Commissioner of Patents, Washington, D.C. 20231 on September 20, 2001

Norris McLaughlin & Marcus, P.A.

By: Yolene Banks

Date 9-20-01

MARKED-UP COPIES OF AMENDED CLAIMS,
SHOWING CHANGES RELATIVE TO PREVIOUS VERSION

Claim 4 (amended). Immunoadsorber according to [Claims 1 to 3] Claim 1, wherein further antibodies against sepsis mediators are contained as a function of the state of the dysregulation.

Claim 5 (amended). Immunoadsorber according to [Claims 1 to 4] Claim 1, wherein these antibodies are aimed against TNF, IL1, IL6, IL8 and/or IL10.

Claim 11 (amended). Immunoadsorber according to [Claims 1 to 10] Claim 1, wherein the organic or synthetic carrier material comprises membranes of particles of polystyrenes, carbohydrates such as cellulose or agarose derivates, or acrylates.

Claim 14 (amended). Method for the production of immunoadsorbers according to [Claims 1 to 13] Claim 1, wherein antibodies aimed against C3a and/or C5a and LPS and, if need be, against further sepsis mediators are covalently or adsorptively coupled to carrier materials of organic or synthetic polymers.

09/937126
JC03 Rec'd PCT/PTO 20 SEP 2001

Immunoadsorber for use in sepsis therapy

The invention in question relates to an immunoadsorber for use in sepsis therapy, in particular for removing complement factors and lipopolysaccharides (LPS) as well as, if need be, TNF and interleukins from body fluids and methods for their production and their use.

Every year, about 3.5 million patients suffer from sepsis in the USA, Japan and the EU. With a total number of inhabitants of 785 million, the incidence for these countries is less than 0.5%. But when hospitalised patients are examined with regard to the frequency of suffering, 2.0 ± 0.16 cases of sepsis are found per 100 hospital admissions. The enormous health political and individual importance can also be seen from the observation that about 25% of these patients also suffer the syndrome of a septic shock, characterised by the lethality rate of >45%, even with most intensive medicinal care by highly qualified specialists in institutions with modern equipment (intensive care units).

The risk of suffering a septic shock is very high especially with poly-traumatised patients (traffic accidents, burns, serious operations). Alongside infection from the outside, breaking through the intestinal barrier for gram-negative bacteria normally occurring in the intestines as a result of a partial loss of function of the immune system of these patients and thus an infection "from the inside" can be detected.

In more than 50% of the cases, gram-negative bacteria or their cell-wall components, endotoxins (lipopolysaccharides, LPS), cause the septic shock. The LPS released by bacteria binds to a serum protein (LBP) and is then absorbed by the LPS receptors of the monocytes/macrophages (CD14). The CD14+ cells activated in this way produce cytokines (TNFα, Interleukin-1 (IL-1), IL-6, IL-8), which have their effect via cytokine receptors of the target cells.

Parallel to the stimulation of the monocytes and macrophages, the complement system is activated. It is an integrated part of the immunological defence of mammals for direct and unspecific combating of bacterial micro-organisms and foreign particles. Of the complement proteins occurring in the blood serum, primarily proenzymes activated by proteolytic fission, the C3 protein with a serum concentration of about 1 g/l plays a central role. After contact of the micro-organisms with the C3,

the complement protein C3a is split off and, on the one hand, the formation of C5 convertase is initiated by the resultant C3b (alternative way of complement activation) and, on the other hand, the reaction is amplified by the C3B converting to C3 convertase due to depositing of serum factors. The complement protein C5 also occurring in serum is now proteolytically fissured by the C5 convertase, which is provided in larger amounts, also forming C5a. Further complement proteins (C6-C9) deposit on the resulting C5b until finally the polymeric hydrophobic membrane attack complex (MAC) is formed, settling in the bacteria membrane (opsonidisation) and forming pores, which lead to phagocytosis and thus to the elimination of the micro-organisms (and the bound MAC). The complement factors C3a and C5a (anaphylatoxins) released in the process of the complement activation result in stimulation of the phagocytising cells to the location of the bacterial attack by increasing the vascular permeability and the release of chemotoxins induced thereby. The reduction of the number of bacteria results in a reduction of the activation of the complement system. This direct and unspecific reaction is closely connected with the other immunological defence systems insofar as the synthesis and release of the cytokines essential for cellular defence is regulated, for example by complement factors. In order to bring about the inflammatory effect, C3a and C5a are bound to specific cell-based receptors, which for their part are expressed in different strengths as a function of the immune reactivity. In order to keep the immune defence permanently ready for activity, activated complement factors are detectable not only after an attack with micro-organisms, but also an integrated part of the serum of standard persons with a concentration of 1 - 10 ng/ml.

The plasma levels of the anaphylatoxins can be increased by a factor of more than one thousand, particularly in a developed sepsis, acute pulmonary failure and in moribund patients.

Almost exclusively on the basis of in vitro examinations, there exist various, mainly unspecifically effective variations of solutions in order to eliminate the effects of various complement factors, which however can hardly be tested under in vivo conditions on account of the side effects to be expected (e.g. WO-A-98/34959).

In ex vivo methods for the prevention of complement activation by artificial, extracorporal surfaces (e.g. surface coatings), an unspecific complement activation was successfully carried out. Further, selective removal of activated complement factors making use of specific C5 antibodies is known from US 5,853,722 and certainly also to be preferred, especially as highly affined antibodies have been generated in the meantime against all the components of the complement system.

The functional cascade manifested is primarily used to eliminate the bacteria penetrating into the organism. But as soon as a discrepancy occurs between the number and/or virulence of the penetrating bacteria and the elimination capacity of the immune system (e.g. in post-traumatic immune deficiency), an excessive activation is observed, subsequently accompanied by a massive release of "shock mediators" (interleukins, thrombocyte activation factor (PAF), but also oxygen radicals, prostaglandins and their metabolic products), thus further limiting the elimination capacity for LPS. In addition, CD14-negative cells (e.g. endotheliae) are also activated by the LPS, as soluble CD14 (sCD14) exists in the blood plasma as an LPS trapper, facilitating binding to these cells and inducing the formation and release of further shock mediators, thus reinforcing the circulus vitiosus. As the shock mediators act selectively, but not specifically, function restrictions in various cells and organs are observed (blood coagulation system, circulation, complement system), with the result that the inflammation reactions attacking the entire organisms initiate shock genesis, leading to irreversible organ damage, to circulation collapse and death.

In order to break through this chain of functions, various therapy strategies have been studied.
Interruption of the cascade with antibodies interrupting the LPS binding to proteins (LBP, sCD14), to the receptor (CD14), to released cytokines or to cytokine receptors or with antagonists blocking the functional areas of the receptors did achieve impressive success in various sepsis models in animal experiments, but there are still no clinically tested, successful prevention and/or therapy studies.

It was not possible to fulfil the high expectations, as it was increasingly seen that LPS also influences and changes the functional condition of cells and tissue which are not impaired by these therapeutic approaches. In addition, it must be taken into account that an LPS (immune complex) inactivated by an antibody/antagonist must be eliminated in order to exclude a biological reactivity on a permanent basis. But the elimination is also a function of the immune system, which, as it is greatly weakened, can hardly or only very incompletely fulfil this task.

The development of the septic shock is a very dynamic occurrence of primarily varying genesis, in which various mediators cause highly differing reactions within a short period of time, these quickly leading to the expression of the septic shock by dysregulation after an initial life-maintaining function.

Therefore, the invention was based on the task of developing an immunoadsorption

system of modular construction, in particular for extra-corporal detoxification, enabling a reduction of the plasma and tissue levels specific to the patient.

Inter alia, the invention is based on the knowledge that TNFα has a key role to play in this regulation system. It is released inter alia by macrophages as a result of various "external" influences such as injuries, inflammations, infections, septicaemia and induces a local and systemic activation of the unspecific and specific defence system via a cytokine cascade (IL-1, IL-6). Clinically, a massive TNFα release is expressed by increased body temperature, lack of appetite and all the subsequent symptoms of a catabolic metabolism situation. In pathogenesis of the sepsis, activation of the macrophages and thus the release of TNFα- appears to be of essential importance for the survival of the patient in the early phase of this disease, whereas the continued state of activation results in the de-compensation of all defence reactions in the further course.

The task of the invention was solved by an immunoadsorber for use in sepsis therapy. The immunoadsorber according to the invention is particularly used for the removal of complement factors and lipopolysaccharides (LPS) as well as the removal of further sepsis mediators, and also TNF and interleukins from body fluids, if need be. It is characterised by carrier materials of organic or synthetic polymers, to which both poly or monoclonal antibodies aimed against the complement factors C3a and/or C5a, and also antibodies aimed against lipopolysaccharides (LPS) are bound. In a preferred embodiment, antibodies aimed against further sepsis mediators are also bound to the carrier.
Preferably, these are polyclonal antibodies, particularly preferably avian antibodies of type IgY. The antibodies against sepsis mediators are contained according to the state of the dysregulation.

According to this invention, these are antibodies aimed against TNF, IL1, IL6, IL8 and/or IL10.

Preferred antibodies against the complement factor C3a manifest specific activity against at least one of the following peptide sequences:

NH_2-KCCEDGMRQNPMR-COOH
NH_2-RFSCQRRTRFISL-COOH
NH_2-ITELRRQHARAS-COOH

Preferred antibodies against the complement factor C5a possess specific activity

against at least one of the following peptide sequences:

NH_2-QADYKDDDDKLPAE-COOH
NH_2-DDKLPAEGLDIENS-COOH

Preferred antibodies against IL1α/β possess specific activity against at least one of the following peptide sequences:

NH_2-NCYSENEEDSSSID-COOH
NH_2-GAYKSSKDDAKIT-COOH
NH_2-WETHGTKNYFTS-COOH
NH_2-RISDHHYSKGFRQA-COOH
NH_2-VQGEESNDKIPVA-COOH
NH_2-ESVDPKNYPKKKMEKRF-COOH

Preferred antibodies against IL6 possess specific activity against at least one of the following peptide sequences:

NH_2-APHRQPLTSSERIDKQI- COOH
NH_2-QNRFESSEEQARA- COOH
NH_2-AITTPDPTTNAS- COOH

Preferred antibodies against IL10 possess specific activity against at least one of the following peptide sequences:

NH_2-SPGQGTQSENSCT-COOH
NH_2-QMKDQLDNLLLKES-CCOH
NH_2-MPQAENQDPDIKA-COOH
NH_2-LPCENKSKAVEQ-COOH

Preferred antibodies against TNFα possess specific activity against at least one of the following peptide sequences:

NH_2-VRSSSRTPSDKPVA-COOH
NH_2-KSPCQRETPEGAEAKPW-COOH

The immunoadsorber according to the invention manifests membranes or particles customary per se of organic or synthetic polymers as carrier materials, e.g. of polystyrenes, carbohydrates such as cellulose or agarose derivatives, or of acrylates, with the specific antibodies being covalently linked to them or fixed to them via

spacers or linkers.

The production of the immunoadsorbers according to the invention is done by methods known per se in that the antibodies aimed against C3a and/or C5a and LPS and, if need be, against further sepsis mediators are coupled convalently or adsorptively to the carrier materials or organic or synthetic polymers.

The specific antibodies are produced by immunisation known per se, preferably of small mammals such as mice, rats or rabbits, or birds, such as chickens, with the corresponding antigens.

The object of the invention is also the use of the immunoadsorbers in appliances for the removal of complement factors, LPS and, if need be, further mediators from body fluids such as blood plasma as a function of the patient-specific situation.

Preferably, the immunoadsorbers are used in sepsis therapy for plasmapherese in patients with sepsis or septic shock.

Although antibodies are available for most substances and are coupled to the various carriers by known methods, avian antibodies are preferably used, as they do not activate the complement system, unlike mammal antibodies. As the activating properties are bound to the F_c part of the mammal antibodies, the F_{ab} fragment fissured with papain can principally also be used.

According to the current state of knowledge, immobilised avian antibodies have no kind of unspecific effects on the human defence system. Birds, preferably chickens, are immunised with customary methods with or without the use of adjuvants. The specific immunoglobulins are excreted in the egg yolk and can be isolated from it with customary methods. They are covalently bound to micro-particles or membranes via the Fc part with known methods.

With the immunoadsorption system for extra-corporal detoxification according to the invention, there exists for the first time a selective system which can be used patient-specifically and by which dysregulations of the immune system can be rectified.

The invention is explained in more detail by the following examples:

Example 1

Production of polyclonal antibodies by means of immunogenic peptides:

The peptides listed in Table 1 are produced by means of a solid phase synthesis:

Table 1

Peptide sequence	**Antigen**
KCCEDGMRQNPMR	C3a
RFSCQRRTRFISL	
ITELRRQHARAS	
QADYKDDDDKLPAE	C5a
DDKLPAEGLDIENS	
SPGQGTQSENSCT	IL10
QMKDQLDNLLLKES	
MPQAENQDPDIKA	
LPCENKSKAVEQ	
NCYSENEEDSSSID	IL1α
GAYKSSKDDAKIT	
WETHGTKNYFTS	
RISDHHYSKGFRQA	IL1β
VQGEESNDKIPVA	
ESVDPKNYPKKKMEKRF	
APHRQPLTSSERIDKQI	IL6
QNRFESSEEQARA	
AITTPDPTTNAS	
VRSSSRTPSDKPVA	TNFα
KSPCQRETPEGAEAKPW	

These peptides are covalently bound to a carrier (KLH) according to a standard recipe. The conjugate dissolved in PBS is mixed in equal shares with Freund's adjuvant. The individual inoculation dose is set in such a way that it contains 200µg of the peptide belonging to the antigen in question. 15-week-old young hens are im-

munised with these mixtures s.c. and boostered 4 times at intervals of 4 weeks.

Example 2

Production of polyclonal antibodies by means of lipopolysaccharides (LPS)

Cleaned LPS (SIGMA) of E. coli J5 are dissolved in PBS and mixed in equal shares with Freund's adjuvant. 15-week-old young hens are immunised with this mixture. The LPS dose amounts to 1 mg of LPS per immunisation. Boostering is done 4 times at intervals of 4 weeks.

Example 3

Obtaining the antibodies (IgY) from egg-yolk:

The eggs from the clutches of the immunised hens are collected. After separation of the egg-yolk containing antibodies, there is storage at –20°C. According to requirements, the yolks are thawed and treated according to the following plan (C.SCHWARZKOPF, B.THIELE (1996) ALTEX 13 Suppl. 16, 35-3):

Solutions

A TBS: 20 mM Tris/HCl, pH 7.5, 0.5 M NaCl
B 10 % (w/v) dextran sulphate in A
C 1 M $CaCl_2$
D 0.5 M EDTA, pH 7.5
E saturated ammonium sulphate solution

The egg yolk (corresponds to a volume of 10 - 20 ml/egg-yolk) is suspended in 100 ml TBS per egg-yolk. Lipids and lipoproteins are precipitated with dextran sulphate (6 ml B per 100 ml TBS/egg yolk suspension) and Ca^{++} (15 ml C per 100 ml TBS-egg yolk suspension), stirred for 30 to 60 min. at room temperature and centrifuged off at 5,000 g. The pellet is washed with a small volume of TBS (approx. 20 mlg/egg yolk) and centrifuged again.
The combined supernatants are filtered through a paper filter, then 0.5 M EDTA is added to the filtrate up to a final concentration of approx. 30 mM EDTA (6 ml per

100 ml), in order to bind remaining Ca^{++} ions. After this, the supernatant is mixed with 24.3 g of ammonium sulphate per 100 ml (corresponds to 40% saturation) and incubated at +4°C for 30 min.. The resultant precipitation (IgY) is firstly washed with 30% $(NH_4)_2SO_4$ (30 ml E + 70 ml dist. water), centrifuged, then dissolved in the smallest possible volume of TBS (approx. 10 ml / egg-yolk used) and dialysed against TBS.
The content of IgY is determined photometrically at 275 nm.

Example 4

a) Activation of a carrier:

The IgY cleaned according to Example 3 are covalently bound to a suitable carrier. For example, sepharose can be activated as described below for this purpose (H.-F.Boeden, W.Büttner, C.Rupprich, D.Büttner, S.Heinrich, M.Becker, M.Holtzhauer (1992) Makromol. Chem. ***193***, 865-887):
The agarose carrier is gradually transformed, i.e. with an amount of acetone increasing in steps of 20%. Finally, the carrier is left to stand in an enclosed container in a quintuple bed volume with water-free acetone overnight, again washed with 5 to 10 Vol. water-free acetone and briefly sucked off on a G2 slice. 400 mg N-(Chlorcarbonyloxy)-5-norbornen-2,3-dicarboximid (ClCOONB) in 10 ml water-free acetone p.a. are added to 10 ml sedimented carrier. Within 15 minutes, a solution of 280 µl triethylamine and 20 mg 4-dimethylamino-pyridine (DMAP) in 5 ml dry acetone is added drop by drop (mol ratio ClCOONB:triethylamine:DMAP 1:1.2:0.1) with shaking. After this, there is further shaking for 15 minutes, after which the carrier is washed with about 200 ml water-free acetone.

b) Coupling of the IgY to a solid carrier

The polysaccharide matrix (gel) activated according to Example 4a) is gradually transformed into a watery medium and then immediately stirred into the coupling solution containing the ligand. Citrate buffer pH 4.2 is used as a coupling buffer. The coupling is done with gentle shaking for 2 h at room temperature. Free bindings are subsequently blocked by addition of ethanolamine. Table 2 shows the concrete conditions for the individual antibodies.

Table 2

Gel No.	Chicken-Ab (IgY)	mg	mg/ml	Ab solution (ml)	ml coupling buffer (Citrate, 0.1 M, pH 4.2)	Ethanolamine 1 M (ml)	moist gel (g)
1	ChalL1	9.5	13.5	0.7	4.3	0.5	5.55
2	ChalL6	9.8	9.8	1.0	4.0	0.5	5.58
3	ChalL10	9.2	7.4	1.2	3.8	0.5	5.55
4	ChaTNF	11.0	11.6	1.0	4.1	0.5	5.56
5	ChaLPS	11.6	13.7	0.9	4.2	0.5	5.60
6	ChaC3a	6.9	10.7	0.6	4.4	0.5	5.57
7	ChaC5a	11.3	11.1	1.0	4.0	0.5	5.55
8	Control	0.0	0.0	0.0	5.0	0.5	5.61

Example 5

The antibodies immobilised according to Example 4 are used in order to remove lipopolysaccharides, interleukins, TNF or complement factors from liquid media such as buffer solutions, serum or blood plasma.

For this, the carriers are washed, transformed into a physiological buffer (PBS) and packed in plastic or glass pillars free of air bubbles. The arrangement is completed by connection to a chromatography appliance. The sample material to be adsorbed (buffer doted with the antigens, serum or blood plasma samples, doted or with natural antigen content) can now be guided by gravity or with a suitable pump via the immobilised antibodies specific for the antigens stated. The existing antigens are recognised, firmly bound and thus removed from the medium flowing through the column by the IgY. The detection of the effectivity is done by analysis (ELISA) of the column throughflow, the antigen content of which is reduced. After washing of the column with a physiological buffer, there is desorption of the bound antigen with suitable elution agents (0.1 M citrate buffer pH 3), fractioning and analysis of the eluate. Quantitative detection of the antigens is used to determine the capacity of the immunosorbent.

Patent claims

1. Immunoadsorber for use in sepsis therapy in which there exist carrier materials of organic or synthetic polymers with bound poly or monoclonal antibodies aimed against the complement factors C3a and/or C5a and against lipopolysaccharides (LPS) and, if need be, with antibodies aimed against further sepsis mediators.

2. Immunoadsorber according to Claim 1, wherein the antibodies are polyclonal antibodies.

3. Immunoadsorber according to Claim 2, wherein the antibodies are avian antibodies of type IgY.

4. Immunoadsorber according to Claims 1 to 3, wherein further antibodies against sepsis mediators are contained as a function of the state of the dysregulation.

5. Immunoadsorber according to Claims 1 and 4, wherein these antibodies are aimed against TNF, IL1, IL6, IL8 and/or IL10.

6. Immunoadsorber according to Claim 1, wherein the bound antibodies are aimed against at least one of the following peptide sequences of the complement factors C3a and C5a

C3a: NH_2-KCCEDGMRQNPMR-COOH
NH_2-RFSCQRRTRFISL-COOH
NH_2-ITELRRQHARAS-COOH

C5a: NH_2-QADYKDDDDKLPAE-COOH
NH_2-DDKLPAEGLDIENS-COOH

7. Immunoadsorber according to Claim 5, wherein the bound antibodies are aimed against at least one of the following peptide sequences of the interleukins 1α and 1β

IL1α: NH2-NCYSENEEDSSSID-COOH

NH_2-GAYKSSKDDAKIT-COOH
NH_2-WETHGTKNYFTS-COOH
IL1β: NH_2-RISDHHYSKGFRQA-COOH
NH_2-VQGEESNDKIPVA-COOH
NH_2-ESVDPKNYPKKKMEKRF-COOH

8 Immunoadsorber according to Claim 5, wherein the bound antibodies are aimed against at least one of the following peptide sequences of interleukin 6

IL6: NH_2-APHRQPLTSSERIDKQI- COOH
NH_2-QNRFESSEEQARA- COOH
NH_2-AITTPDPTTNAS- COOH

9. Immunoadsorber according to Claim 5, wherein the bound antibodies are aimed against at least one of the following peptide sequences of interleukin 10

IL10: NH_2-SPGQGTQSENSCT-COOH
NH_2-QMKDQLDNLLLKES-CCOH
NH_2-MPQAENQDPDIKA-COOH
NH_2-LPCENKSKAVEQ-COOH

10. Immunoadsorber according to Claim 5, wherein the bound antibodies are aimed against at least one of the following peptide sequences

TNFα: NH_2-VRSSSRTPSDKPVA-COOH
NH_2-KSPCQRETPEGAEAKPW-COOH

11. Immunoadsorber according to Claims 1 to 10, wherein the organic or synthetic carrier material comprises membranes of particles of polystyrenes, carbohydrates such as cellulose or agarose derivatives, or acrylates.

12. Immunoadsorber according to Claims 1 to 11, wherein the specific antibodies are covalently bound to the membranes or particles.

13. Immunoadsorber according to Claims 1 to 11, wherein the antibodies are fixed to the carrier materials via spacers or linkers.

14. Method for the production of immunoadsorbers according to Claims 1 to 13, wherein antibodies aimed against C3a and/or C5a and LPS and, if need be, against further sepsis mediators are covalently or adsorptively coupled to carrier materials of organic or synthetic polymers.

15. Method according to Claim 14, wherein the antibodies are produced by immunisation preferably of small mammals, such as mice, rats or rabbit, or of birds, such as chickens, with the corresponding antigens.

16. Use of immunoadsorbers according to Claims 1 to 13 as an effective component of an appliance for the removal of complement factors, LPS and, if need be, further mediators in a patient-specific combination of body fluids.

17. Use according to Claim 16, wherein the immunoadsorbers are used for plasmapherese in patients with sepsis or septic shock as well as other diseases connected with inflammations.

Abstract

The invention relates to immunoadsorbers for use in sepsis therapy, in particular for removal of complement factors and lipopolysaccharides (LPS) and, if need be, further sepsis mediators such as TNF and interleukins from body fluids, methods for their production and their use.

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If each inventor understands English, the Declaration and Power of Attorney below is suitable for use when filing a regular patent application and also when entering the national stage, in the case of an International application designating the USA under the PCT.

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION	Attorney Docket No. 101195-63

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name,
I believe I am the original, first and sole inventor (if only one name is listed below at 201) or an original, first and joint inventor (if plural names are listed below at 201-205) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Immunoadsorber for Use in Sepsis Therapy

the specification of which (check one)

____ is attached hereto

__✓__ was filed on ____23 March 2000_____

under Serial Number __PCT/DE00/00927__ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I list below any prior foreign application(s) for patent or inventor's certificate in respect of which foreign priority benefits are claimed under 35 USC 119; and any prior foreign application(s) for patent or inventor's certificate in respect of which such foreign priority rights are not claimed and which has a filing date before that of any application in respect of which such foreign priority benefits are claimed:

Application Number	Country	Filing Date (day, month, year)	Priority Claimed under 35 USC 119
199 13 707.2	**Germany**	**26 March 1999**	YES:__✓_ NO:____
			YES:____ NO:____
			YES:____ NO:____

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No.	Filing Date

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201	Date 11/07/01
Signature of Inventor 202	Date 7.11.2001
Signature of Inventor 203	Date 12.11.2001
Signature of Inventor 204	Date 09.11.2001
Signature of Inventor 205	Date 26.11.2001

SEQUENCE LISTING

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